specific reaction at a negligible level. EmA9/EmA9 sELISA had an increased non-specific reactivity, but was improved by using lower antibody concentration.

Furthermore, a Dot-ELISA, in which a nitrocellulose membrane coated with capture antibody, biotinylated antibody as primary antibody and streptavidin biotinylated horseradish peroxidase (HRP) complex were used to visualize the presence of the antigens, was developed for 'on the spot' diagnostic method in field survey situation. This method using EmA9/EmA9 system gave a slightly lower sensitivity compared to sELISA using plates, and showed to be useful as a simple diagnostic method.

It is concluded that the improved sensitivity of the detection of *E. multilocularis* coproantigens was achieved by the application of ABC methods, which lead to the development of a simple diagnostic method based on a Dot-ELISA for use in the field.

Time course of antibody response against the infection with eggs of *Echinococcus multilocularis* in mice.

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Although *Echinococcus* have been recognized as one of the causative agents of most serious parasitic zoonosis, information on the intermediate hosts infected via ingestion of eggs is poorly available. This is attributed by the limitation of the availability of safe animal laboratory facilities able to handle those eggs. In this study, experimental infection was done using viable *E. multilocularis* eggs and the time course of antibody response against the infection with eggs was evaluated in susceptible mouse strain (AKR/N).

A total of 35 mice were orally inoculated with approximately 300 parasite eggs. The mice were necropsied at 1, 2, 4, 6, 9, 12 and 16 weeks post-infection (WPI). Histological investigation of the parasite development showed the formation of laminated layer at 4 WPI, formation of brood capsule with the massive increase of germinal cells at 9 WPI and formation of protoscoleces at 16 WPI.

The antibody responses of mice to three parasite antigens prepared from immature and mature metacestodes and purified protoscoleces were evaluated by ELISA and immunoblotting. In ELISA, the time courses of antibody response to three antigens were similar, in which the increase of OD value was initially detected at 4WPI and was more apparent at 9 WPI. The observation indicated that a part of antigen molecules expressed in protoscoleces were also expressed in immature metacestode even before the formaion of protoscoleces. Comparison of the band pattern in immunoblotting of the antigens showed several common bands in these antigens. This also indicates that immature metacestode had already expressed the antigen molecules expressed in protoscoleces. Immunoblot using sera collected at various WPI showed that many of bands appeared from 4 or 9 WPI. The result indicated that host antibody response is strongly stimulated by the prolifera-

tion of germinal cells and the formation of brood capsules.

In an immunohistological study using the sections of immature and mature metacestodes with various WPI sera, brood capsule and germinal layer, which is composed of germinal cells, was stained heavily besides the surface and parenchyma of protoscoleces, and calcareous corpuscles. This finding supports the result of ELISA, and the host antibody response was strongly stimulated by the increased proliferation of germinal cell which was required for the fomation of brood capsules and germinal layers.

In ELISA using a polysaccharide antigen extracted from mature metacestode, the OD value suddenly increased at 9 WPI. It is suggested that the antigen expression increased dramatically during this period, and induced the strong antibody response.

It was concluded that antibody response was greatly stimulated by the active proliferations of the germinal cells which were observed in brood capsule and protoscoleces formation during 9 to 16 WPI.

The findings in this study contribute to the establishment of serodiagnostic methods for detection of alveolar echinococcosis in domestic and wild animals as well as in humans.

Ecological study on the hookworm, *Uncinaria lucasi*, of northern fur seal, *Callorhynus ursinus*, in bering island, russia

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In July and August, 1995, a survey was performed on pups of northern fur seals (*Callorhynus ursinus*) for the hookworm (*Uncinaria lucasi*) infection in Northwestern Rookery in Bering island, Kommandorski Islands, Russia. *U. lucasi* were collected from the intestines of 26 dead pups in the rookery and its biological characteristics was determined. Moreover, day age estimation of 23 pups was performed using their teeth and the relationship of the parasite development, burden and distribution in the intestine and age of seals were compared.

U. lucasi were found in 84.6% (22/26) of the dead pups. Among 23 pups performed age estimation, larva were found in 50% of the pups with 3–10 days of age and adult parasites were in 100% (15/15) with 15–35 days of age. Because the pathogenicity of larval *U. lucasi* is weak, only 50% of the pups with less than 10 days old were parasitized and the external traumas were recognized on their bodies, it is predicted that the mortality of those pups was not due to the parasite infection. On the other hand, all the dead pups with 15–35 days old harbored numerous adult *U. lucasi* which are pathogenic to the hosts. In these pups, 2 of them harbored less than 800 worms, 5 with 800 to 1,600 and 8 with more than 1,600. Therefore, it is predicted that the mortality of those pups was due to the large number of infection with adult *U. lucasi*, although the possibility of other causes of the mortality can not be denied. In the individual pups, *U. lucasi* was most often found in the middle of the small intestine. Because no difference was observed in the larvae and adult distribution in the intestines of those pups, it is suggested that *U. lucasi*